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Synthesis and antiproliferative activity in vitro of novel (2-butynyl)thioquinolines

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ABSTRACT

The series of new acetylenic thioquinolines containing propargyl, 2-butynyl, 4-bromo-2-butynyl, and 4-hydroxy-2-butynyl groups has been prepared and tested for antiproliferative activity in vitro against human [SW707 (colorectal adenocarcinoma), CCRF/CEM (leukemia)] and murine [P388 (leukemia), B16 (melanoma)] cancer lines. All the compounds obtained exhibited antiproliferative activity. The most active compounds **7, 16, 17**, and **19** have the ID_{50} values ranging from 0.2 to 4.6 μg/ml comparable to that of cisplatin used as referencecompounds.

1. Introduction

Acetylenic derivatives are an important class of compounds that have attracted increasing attention as a source of new anticancer agents. The synthetic methods for their preparation are of interest especially with regard to the synthesis of enediyne antitumor antibiotics such as calicheamicin, esperamycin, dynemicin, namenamicin, shishijimicin, and uncialamycin or similar molecules models.[1–5] The natural enediynes are the most potent of anticancer agents discovered, some members of which are three order of magnitude more potent than other anticancer drugs but their clinical use has been limited because of their toxicity and modest selectivity for cancer cells. It has prompted several research groups to design, prepare, and test new simplified, fully synthetic acetylenic analogues, characterized by a similar mode of action. Several cyclic and acyclic models have recently been developed, some of them including pyridine and quinoline units.[6–12]

We have reported a simple and efficient method for the synthesis of thioquinolines, which possess O, S, Se-propargyl groups.[13–15] The new propargyl thioquinolines obtained exhibit antiproliferative activity in vitro against a broad panel of human and murine cancer cell lines. The most cytotoxic compounds of these series 4-(3-hydroxypropoxy)-3′-propargylthio-3,4′-diquinoline sulfide, and 3-methylthio-4-propargylselenoquinoline approached the activity of cisplatin.[13–15] It seems most likely that a propargyl group may be essential for antiproliferative activity of these compounds. The structure–activity relationships study show a significant correlation between the antiproliferative activity and the electronic properties expressed as ^{13}C NMR chemical shift and lipophilicity.[14,16,17]

As an extension of our work on the development of anticancer drugs, we synthesized derivatives **7–24** possessing propargyl, 2-butynyl, 4-bromo-2-butynyl, and 4-hydroxy-2-butynyl groups with the aim to obtain more information about the influence of substituents on antiproliferative activity in this class of compounds.

2. Results and discussion

2.1. Chemistry

The synthesis of acetylenic thioquinolines **7–24** (Scheme 2) was accomplished starting with 4-chloro-3-methylthio-quinoline **3** or 4-chloro-3-propargylthioquinoline **4** or 4-chloro-3-(2-butynylthio)quinoline **5** and 4-chloro-3-(4-hydroxy-2-butynylthio)quinoline **6**.

Compounds **3–5** were prepared according to our previously reported methods.[13,14,18] 4-Chloroquinoline **6** was synthesized as shown in Scheme 1. The starting **1** was prepared according to our published procedure.[18] Treatment of **1** with sodium methoxide in DMSO at 20 °C give sodium 4-chloro-3-quinolinethiolate **1-A** and 4-methoxy-3-methylthioquinoline **2**, which was removed by extraction. Sodium salt **1-A** after S-alkylation using 1-bromo-4-hydroxy-2-butyne gave with 65% yield **6**.

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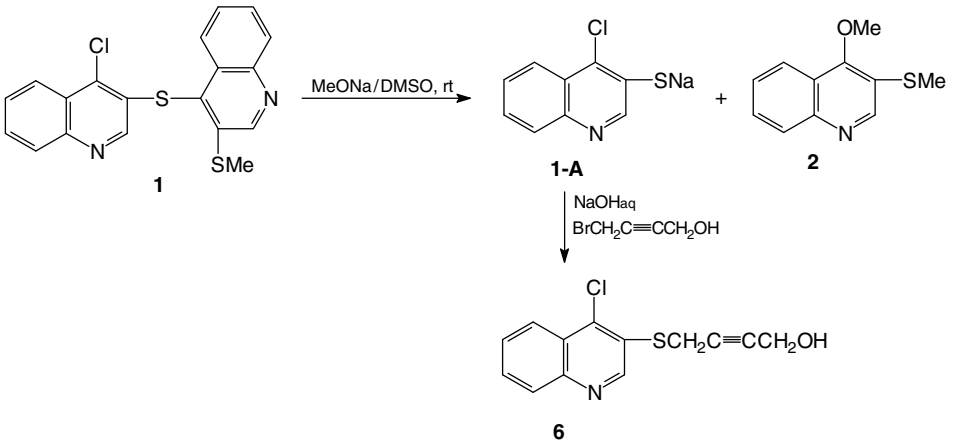
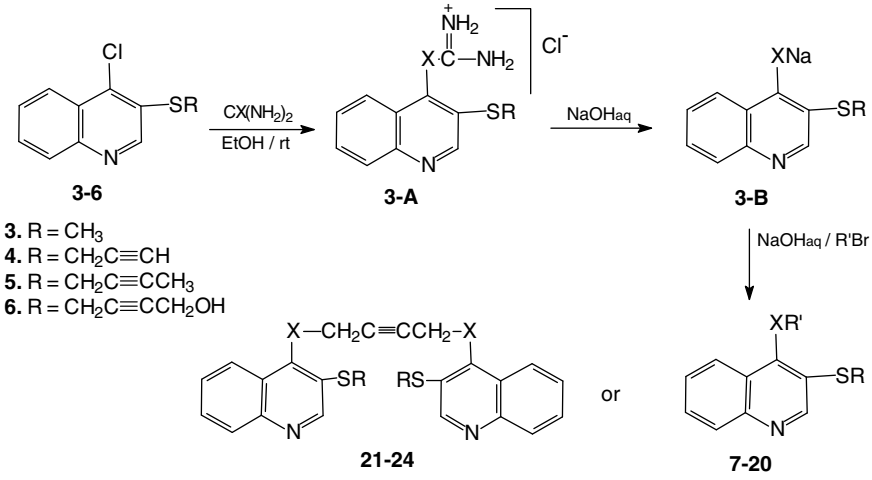


Scheme 1. Synthesis of 4-chloro-3-(4-hydroxy-2-butynylthio)quinoline **6**.

Compounds **3–6** were converted into **9–15** and **17–24** by nucleophilic displacement of chlorine atom by thiourea or selenourea in ethanol, hydrolysis of uronium salt **3-A** and subsequent S- or Se-alkylation of sodium salt **3-B** with methyl iodide or propargyl bromide or 1,4-dibromo-2-butyne or 1-bromo-4-hydroxy-2-butyne (Scheme 2). In the case of the synthesis of compounds **7**, and **16** the solution of sodium salt **3-B**, which was obtained from the reaction of corresponding **4** or **6** with thiourea or selenourea, was added to the ethanol solution containing an excess of 1,4-dibromo-2-butyne. Under these conditions, the concentration of **3-B** will always is low and it will favor the formation of corresponding 4-bromo-2-butynyl derivatives **7, 8** and **16**. The crude products were isolated from aqueous sodium hydroxide by filtration or extraction and separated by column chromatography.

2.2. Antiproliferative activity

The eighteen compounds were tested in SRB or MTT (in the case of leukemia cells) assay for their antiproliferative activity in vitro against two human cancer cell lines: SW707 (colorectal adenocarcinoma), CCRF/CEM (leukemia) and two murine cancer cell lines: P388 (leukemia), B16 (melanoma). The results of cytotoxic activity in vitro were expressed as an ID_{50} (μg/ml), that is, the concentration of compound, which inhibits the proliferation of 50% of tumor cells as compared to the control untreated cells. Cisplatin was



3. R = CH_3
4. R = $CH_2C{\equiv}CH$
5. R = $CH_2C{\equiv}CCH_3$
6. R = $CH_2C{\equiv}CCH_2OH$

21. R = $CH_2C{\equiv}CH$, X = S
22. R = $CH_2C{\equiv}CCH_3$, X = S
23. R = (3) $CH_2C{\equiv}CCH_3$, X = (4) Se
R = (3') $CH_2C{\equiv}CH$, X = (4') S
24. R = CH_3, X = S

7. R = $CH_2C{\equiv}CCH_2OH$, R' = $CH_2C{\equiv}CCH_2Br$, X = Se
8. R = $CH_2C{\equiv}CCH_2OH$, R' = $CH_2C{\equiv}CCH_2Br$, X = S
9. R = $CH_2C{\equiv}CCH_2OH$, R' = $CH_2C{\equiv}CCH_2OH$, X = Se
10. R = $CH_2C{\equiv}CCH_2OH$, R' = $CH_2C{\equiv}CCH_2OH$, X = S
11. R = $CH_2C{\equiv}CCH_2OH$, R' = CH_3, X = Se
12. R = $CH_2C{\equiv}CCH_2OH$, R' = CH_3, X = S
13. R = $CH_2C{\equiv}CCH_2OH$, R' = CH_3, X = O
14. R = $CH_2C{\equiv}CCH_2OH$, R' = $CH_2C{\equiv}CH$, X = Se
15. R = $CH_2C{\equiv}CCH_2OH$, R' = $CH_2C{\equiv}CH$, X = S
16. R = $CH_2C{\equiv}CH$, R' = $CH_2C{\equiv}CCH_2Br$, X = Se
17. R = $CH_2C{\equiv}CH$, R' = $CH_2C{\equiv}CCH_2OH$, X = Se
18. R = $CH_2C{\equiv}CH$, R' = $CH_2C{\equiv}CCH_2OH$, X = S
19. R = CH_3, R' = $CH_2C{\equiv}CCH_2OH$, X = Se
20. R = CH_3, R' = $CH_2C{\equiv}CCH_2OH$, X = S

Scheme 2. Synthesis of acetylenic thioquinolines **7–24**.

applied as a referential cytotoxic agent (positive test control). A value of less than 4 μg/ml is considered as an antiproliferative activity criterion for synthetic compounds. The results of the cytotoxicity studies are summarized in Table 1.

In general all the compounds obtained exhibited a potent antiproliferative activity against human and murine cancer lines applied. 4-Chloro-3-(4-hydroxy-2-butynylthio)quinoline **6** exhibited high activity against P388 and CCRF/CEM and no antiproliferative activity against B16. As reported previously 4-chloro-3-(2-butynylthio)quinoline possessed lower cytotoxic activity than **6**. It indicated that 4-hydroxy-2-butynylthio group slightly influenced the activity than 2-butynylthio group. The substitution of chlorine atom of **6** by 4-bromo-2-butynylseleno group, compound **7**, resulted in increase of activity against all cancer lines applied, especially against the cells of B16 melanoma. For example compound **7** was 20-fold more cytotoxic than **6** against CCRF/CEM and 2-fold more active than cisplatin against CCRF/CEM leukemia. Its sulfur analogue **8** was quite active as **7** against CCRF/CEM and B16 cells, but less active against SW707 and P388.

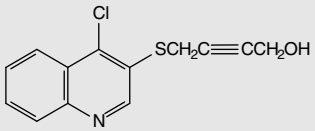
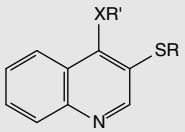
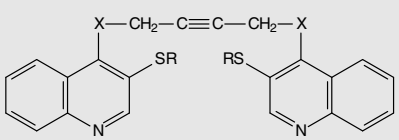
The replacement of 4-bromo-2-butynyl group by 4-hydroxy-2-butynyl group, compounds **9–10**, resulted in decrease of activity, especially against the cells of SW707 in the case of selenium analogue **9** and against B16 in the case of **10**. On the other hand, the activity of compound **10** against P388 was higher than that observed for **8**.

In the series of derivatives **11–13** containing Se-, S-, and O-methyl groups, the selenium compound **11** showed high activity toward SW707, CCRF/CEM, and B16 cells in the concentration range applied but was less active against P388. The replacement of selenium atom by sulfur or oxygen, compounds **12** and **13**, respectively, resulted in decrease of activity, especially against the cells of B16 melanoma.

Among compounds **14–15**, the selenium derivative **14** was more active than sulfur analogue **15** except the activity against of CCRF/CEM cells.

In the series of compounds **16–18** containing thiopropargyl group at position 3, the selenium compound **16** showed the most potent activity with the ID_{50} values comparable to that of compound **7**. These results may suggest that 4-bromo-2-butynyl group significantly affects the cytotoxic activity of the studied compounds. It is important to note that **17** exhibited the same activity against four cancer cells lines applied with ID_{50} values in the range 3.1–3.6 μg/ml. The replacement of selenium atom by sulfur, compound **18**, led to the considerable decrease of activity against the cells of SW707 and B16 but retained activity toward two other cancer cell lines used. A similar change of cellular response was also

Table 1
Structures of acetylenic thioquinolines **7**–**24** and their antiproliferative activity in vitro and referential cisplatin against the cells of human and murine cancer cell lines

Compound				Antiproliferative activity ID_{50} [μg/ml]			
				Human		Murine	
				SW707	CCRF/CEM	P388	B16
6				6.2 ± 1.1	4.1 ± 0.3	2.4 ± 0.0	Neg
							
	R	R′	X				
7	$CH_2C{\equiv}CCH_2OH$	$CH_2C{\equiv}CCH_2Br$	Se	2.8 ± 0.4	0.2 ± 0.1	1.6 ± 1.2	4.6 ± 1.3
8	$CH_2C{\equiv}CCH_2OH$	$CH_2C{\equiv}CCH_2Br$	S	36.2 ± 4.5	0.6 ± 0.6	43.2 ± 14.1	4.9 ± 1.0
9	$CH_2C{\equiv}CCH_2OH$	$CH_2C{\equiv}CCH_2OH$	Se	26.6 ± 6.1	1.4 ± 1.2	4.1 ± 1.7	4.3 ± 1.1
10	$CH_2C{\equiv}CCH_2OH$	$CH_2C{\equiv}CCH_2OH$	S	32.0 ± 2.9	2.6 ± 0.4	5.7 ± 0.8	42.2 ± 6.5
11	$CH_2C{\equiv}CCH_2OH$	Me	Se	3.5 ± 0.3	2.1 ± 1.5	19.8 ± 10.4	5.8 ± 2.2
12	$CH_2C{\equiv}CCH_2OH$	Me	S	69.3 ± 7.7	13.9 ± 8.2	32.3 ± 11.5	Neg
13	$CH_2C{\equiv}CCH_2OH$	Me	O	67.4 ± 6.6	22.0 ± 2.2	23.2 ± 10.1	Neg
14	$CH_2C{\equiv}CCH_2OH$	$CH_2C{\equiv}CH$	Se	3.8 ± 0.3	1.9 ± 1.2	2.5 ± 0.8	6.8 ± 4.7
15	$CH_2C{\equiv}CCH_2OH$	$CH_2C{\equiv}CH$	S	Neg	2.7 ± 1.3	13.3 ± 0.5	Neg
16	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_2Br$	Se	2.2 ± 1.4	0.3 ± 0.1	1.2 ± 1.3	4.2 ± 0.4
17	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_2OH$	Se	3.3 ± 0.2	3.2 ± 1.2	3.1 ± 0.3	3.6 ± 0.6
18	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CCH_2OH$	S	42.6 ± 15.0	3.2 ± 0.9	4.0 ± 0.2	54.7 ± 2.7
19	Me	$CH_2C{\equiv}CCH_2OH$	Se	3.4 ± 0.1	0.5 ± 0.3	2.6 ± 1.1	4.2 ± 0.6
20	Me	$CH_2C{\equiv}CCH_2OH$	S	34.9 ± 7.6	2.3 ± 0.7	3.1 ± 0.2	49.8 ± 0.5
							
	R		X				
21	$CH_2C{\equiv}CH$		S	Neg	Neg	Neg	Neg
22	$CH_2C{\equiv}CCH_3$		S	Neg	Neg	Neg	Neg
23	(3) $CH_2C{\equiv}CCH_3$ (3′) $CH_2C{\equiv}CH$		(4)Se (4′)S	43.0 ± 5.2	30.3 ± 10.6	30.7 ± 8.9	Neg
24	Me		S	Neg	Neg	Neg	Neg
Cisplatin				3.5 ± 0.7	0.4 ± 3.3	2.6 ± 1.4	4.5 ± 1.1

Neg—negative in the concentration used.

observed for **9–12, 14–15, 19–20**, and other compounds previously reported.[15]

The antiproliferative activities of two compounds **19–20** were similar to **17–18**. This indicates that thiomethyl and thiopropargyl substituents at position 3 had a similar effect on the cytotoxicity of these compounds.

A structure–activity relationship observed in compounds **7, 9, 11**, and **14** indicated that the cytotoxic activity against SW707 was in the order 4-bromo-2-butynyl > methyl > propargyl > 4-hydroxy-2-butynyl. Whereas, the activity of these compounds against B16 was as follows: 4-hydroxy-2-butynyl > 4-bromo-2-butynyl > methyl > propargyl. In general, among the compounds prepared in this study, **7** and **16** exhibited the most potent cytotoxicity with ID_{50} values <2.8 μg/ml against cancer cell lines including SW707, CCRF/CEM, and P388. These results may suggest that bromobutynyl function is important for anti-leukemia/colorectal activity. While the compounds **17** and **9** were most active against B16 melanoma. In this cancer subtype, it seemed that the 4-hydroxy-2-butynyl group was the most suitable substituent for eliciting better anti-melanoma activity.

Considering the overall activities of **7–20** it can be postulated that substitution of selenium (X=Se) with sulfur (X=S) reduces the cytotoxic activity. Another noteworthy feature of the obtained compounds results in the observation that melanoma cells (B16) appear to be relatively resistant to the cytotoxic effects of the studied compounds as compared to of the three other cancer cells lines applied. On the other hand, the leukemia cancer cells (CCRF/CEM) appear to be more sensitive to the cytotoxic effects of compounds **7–20**.

1,4-Disubstituted butynes **21-24** do not show any significant activity.

3. Conclusions

Novel acetylenic thioquinolines **7–24**, possessing in positions 3 and 4, one or two, propargyl, 2-butynyl, 4-bromo-2-butynyl, and 4-hydroxy-2-butynyl groups were synthesized in good yields using 4-chloro-quinoline derivatives **3–6** as starting material. The obtained compounds were evaluated for antiproliferative activity in vitro against two human cancer cell lines: SW707 (colorectal cancer), CCRF/CEM (leukemia) and two murine cancer cell lines: P388 (leukemia), B16 (melanoma). All tested compounds showed varied activity against different cancer cell lines. As a result of the SAR, it was revealed that the nature of the acetylenic substituent at the C-3 and C-4 positions and character of the heteroatoms (Se and S) at C-4 critically influence the anticancer activity in vitro of the study compounds. Among the prepared compounds, **7, 16, 17**, and **19** were found to be the most active, with ID_{50} values ranging from 0.2 to 4.6 μg/ml comparable to that of referential anticancer drug, cisplatin. These compounds seem to be good candidate for further anticancer activity studies in vitro using a broad panel of human and murine cell lines with the aim to selected compounds for studies in vivo.

4. Experimental

4.1. General techniques

Melting points were determined in open capillary tubes on a Boetius melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz) spectra were recorded on a Bruker MSL 300 spectrometer in $CDCl_3$ or DMSO-d_6 solvents with tetramethylsilane as internal standard; chemical shifts are reported in ppm (δ) and J values in Hz. Multiplicity is designated as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad singlet (br s). Mass spectra were recorded under +CI conditions on Finnigan MAT 95 using isobutane as a reagent and temperature of ion source of 200 °C. FAB MS spectra were recorded on Finnigan MAT 95 spectrometer in +FAB mode (Cs^+, 13 keV, nba). Elemental C, H, N analyses were obtained on a Carlo Erba Model 1108 analyzer. TLC was performed on silica gel 60 254F plates (Merck) using a mixture of chloroform and ethanol (15:1, v/v) as an eluent. UV light and iodine accomplished visualization. Column chromatography was performed on silica gel 60, <63 μm (Merck) using a mixture of chloroform and ethanol (30:1, v/v) as an eluent. Solvents were dried and purified according to literature procedures.

4.2. Chemistry

The starting compounds: 4-chloro-3′-methylthio-3,4′-diquinolinyl sulfide **1**,[18] 4-chloro-3-(methylthio)-quinoline **3**,[18] 4-chloro-3-propargylthio-quinoline **4**,[13] 4-chloro-3-(2-butynylthio)quinoline **5**,[14] 1,4-dibromo-2-butyne,[19] 1-bromo-4-hydroxy-2-butyne[20] were obtained according to methods described previously.

4.2.1. Synthesis of 4-chloro-3-(4-hydroxy-2-butyny-lthio)-quinoline (6)

A mixture of 4-chloro-3′-methylthio-3,4′-diquinolinyl sulfide **1** (0.74 g, 2 mmol) and sodium methoxide (0.32 g, 6 mmol) in 8 mL DMSO was stirred at room temperature for 30 min. The reaction mixture was poured into 20 ml of 5% aqueous sodium hydroxide and extracted with 4 × 5 mL of chloroform. The combined extracts were washed with water, dried with anhydrous magnesium sulfate and evaporated to give crude **2**. To the water layer 1-bromo-4-hydroxy-2-butyne (0.30 g, 2 mmol) was added and stirred for 30 min. The mixture was extracted with 4 × 5 mL of chloroform. The combined organic layer was washed with water and dried with anhydrous magnesium sulfate. After removal of the solvent the residue was purified by column chromatography to give 0.32 g (65%) pure product **6**: mp 120–122 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 4.03 (t, J = 2.4 Hz, 2 H, SCH_2), 4.25 (t, J = 2.4 Hz, 2H, CH_2OH), 7.76–7.86 (m, 2H, H-6 and H-7), 8.08–8.19 (m, 2H, H-5 and H-8), 8.99 (s, 1H, H-2). CI MS m/z (rel. intensity) 266 ($M+2+H^+$, 43), 264 ($M+H^+$, 100). Anal. Calc. for $C_{13}H_{10}ClNOS$: C 59.20, H 3.82, N 5.31. Found: C 59.31, H 3.79, N 5.42.

4.2.2. General procedure for the synthesis of acetylenic thioquinolines 7–24

A mixture of 4-chloro-3-methylthioquinoline **3** (0.42 g, 2 mmol) or 4-chloro-3-propargylthioquinoline **4** (0.45 g, 2 mmol) or 4-chloro-3-(2-butynylthio)quinoline **5** (0.49 g, 2 mmol) or 4-chloro-3-(4-hydroxy-2-butynylthio)quinoline (0.50 g, 2 mmol) **6** and selenourea (0.26 g, 2.1 mmol) or thiourea (0.16 g, 2.1 mmol) in 99.8% ethanol (8 mL) was stirred at room temperature for 1 h. The reaction mixture was poured into 20 mL of 5% aqueous sodium hydroxide. Methyl iodide (0.31 g, 2.2 mmol) or propargyl bromide (0.26 g, 2.2 mmol) or 1,4-dibromo-2-butyne (0.25 g, 1.1 mmol) or 1-bromo-4-hydroxy-2-butyne (0.34 g, 2.3 mmol) was added dropwise to the aqueous layer, and the mixture was stirred for 15 min. The resultant solid was filtered off, washed with water and air-dried to give crude products **9–15, 17–20**, or **21–24**. Compounds **7–8** and **16** were obtained when aqueous layer containing sodium salt **3-B** was added dropwise to the excess of 1,4-dibromo-2-butyne (0.89 g, 4.2 mmol) in 1 mL ethanol. Obtained crude products were separated by column chromatography and then crystallized from a mixture of benzene and hexane to give pure products **7–24**.

4.2.2.1. 4-(4-Bromo-2-butynylseleno)-3-(4-hydroxy-2-butynylthio)quinoline (7). Yield 56%; mp 102–103 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.65–3.67 (m, 4H, $SeCH_2$ and CH_2Br), 3.86 (t,

J = 2.1 Hz, 2H, SCH_2), 4.24 (t, J = 2.1 Hz, 2H, CH_2OH), 7.62–7.74 (m, 2H, H-6 and H-7), 8.07–8.53 (m, 2H, H-5 and H-8), 8.97 (s, 1H, H-2). FAB MS (+VE) m/z (rel. intensity) 442 (M+2+H$^+$, 24), 440 (M+H$^+$, 36). Anal. Calc. for $C_{17}H_{14}BrNOSSe$: C 46.49, H 3.21, N 3.19. Found: C 46.40, H 3.23, N 3.31.

4.2.2.2. 4-(4-Bromo-2-butynylthio)-3-(4-hydroxy-2-butynylthio)quinoline (8). Yield 47%; mp 90–91 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.64 (t, J = 2.1 Hz, 2H, CH_2Br), 3.89 (t, J = 2.1 Hz, 2H, SCH_2), 3.75 (t, J = 2.1 Hz, 2H, SCH_2), 4.25 (t, J = 2.1 Hz, 2H, CH_2OH), 7.63–7.75 (m, 2H, H-6 and H-7), 8.08–8.57 (m, 2H, H-5 and H-8), 9.00 (s, 1H, H-2). FAB MS (+VE) m/z (rel. intensity) 391 (M+H$^+$, 8), 279 (100). Anal. Calc. for $C_{17}H_{14}BrNOS_2$: C 52.04, H 3.60, N 3.57. Found: C 52.22, H 3.53, N 3.41.

4.2.2.3. 3-(4-Hydroxy-2-butynylthio)-4-(4-hydroxy-2-butynylseleno)quinoline (9). Yield 43%; mp 81–83 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.63 (t, J = 2.1 Hz, 2H, SCH_2), 3.86 (t, J = 2.1 Hz, 2H, $SeCH_2$), 3.99 (t, J = 2.1 Hz, 2H, CH_2OH), 4.22 (t, J = 2.1 Hz, 2H, CH_2OH), 7.60–7.73 (m, 2H, H-6 and H-7), 8.05–8.50 (m, 2H, H-5 and H-8), 8.96 (s. 1H. H-2). CI MS m/z (rel. intensity) 378 (M+H$^+$, 100). Anal. Calc. for $C_{17}H_{15}NO_2SSe$: C 54.26, H 4.02, N 3.72. Found: C 54.12, H 4.11, N 3.65.

4.2.2.4. 3-(4-Hydroxy-2-butynylthio)-4-(4-hydroxy-2-butynylthio)quinoline (10). Yield 58%; mp 119–121 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 3.81 (t, J = 2.1 Hz, 2H, SCH_2), 3.87 (br s, 2H, CH_2OH), 4.04 (br s, 2H, CH_2OH), 4.16 (t, J = 2.1 Hz, 2H, SCH_2), 7.67–7.79 (m, 2H, H-6 and H-7), 8.02–8.49 (m, 2H, H-5 and H-8), 8.97 (s, 1H, H-2). CI MS m/z (rel. intensity) 330 (M+H$^+$,100). Anal. Calc. for $C_{17}H_{15}NO_2S_2$: C 61.98, H 4.59, N 4.25. Found: C 61.79, H 4.63; N 4.36.

4.2.2.5. 3-(4-Hydroxy-2-butynylthio)-4-methylselenoquinoline (11). Yield 42%; mp 119–120 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.35 (s, 3H, $SeCH_3$), 3.85 (t, J = 2.1 Hz, 2H, SCH_2), 4.24 (t, 2H, CH_2OH), 7.59–7.72 (m, 2H, H-6 and H-7), 8.05–8.48 (m, 2H, H-5 and H-8), 8.92 (s, 1H, H-2). CI MS m/z (rel. intensity) 324 (M+H$^+$, 100). Anal. Calc. for $C_{14}H_{13}NOSSe$: C 52.18, H 4.07, N 4.35. Found: C 52.29, H 4.01, N 4.41.

4.2.2.6. 3-(4-Hydroxy-2-butynylthio)-4-methylthioquinoline (12). Yield 64%; mp 102–103 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.47 (s, 3H, SCH_3), 3.87 (t, J = 2.1 Hz, 2H, SCH_2), 4.24 (br s, 2H, CH_2OH), 7.61–7.73 (m, 2H, H-6 and H-7), 8.06–8.52 (m, 2H, H-5 and H-8), 8.95 (s, 1H, H-2). FAB MS (+VE) m/z (rel. intensity) 276 (M+H$^+$, 100). Anal. Calc. for $C_{14}H_{13}NOS_2$: C 61.06, H 4.76, N 5.09. Found: C 60.87, H 4.89, N 4.88.

4.2.2.7. 3-(4-Hydroxy-2-butynylthio)-4-methoxyquinoline (13). Yield 55%; mp 57–58 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: *3.71 (t,* J = 2.1 Hz, 2H, SCH_2), 4.19–4.23 (m, 5H, OCH_3 and CH_2OH), 7.57–7.73 (m, 2H, H-6 and H-7), 8.05–8.12 (m, 2H, H-5 and H-8), 8.95 (s, 1H, H-2). CI MS m/z (rel. intensity) 260 (M+H$^+$, 100). Anal. Calc. for $C_{14}H_{13}NO_2S$: C 64.84, H 5.05, N 5.40. Found: C 64.67, H 5.09, N 5.38.

4.2.2.8. 3-(4-Hydroxy-2-butynylthio)-4-(propargylseleno)-quinoline (14). Yield 57%; mp 89–90 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.12 (t, J = 2.7 Hz, 1H, CH), 3.60 (d, J = 2.7 Hz, 2H, $SeCH_2$), 3.85 (t, J = 2.1 Hz, 2H, SCH_2), 4.23 (t, J = 2.1 Hz, 2H, CH_2OH), 7.60–7.74 (m, 2H, H-6 and H-7), 8.06–8.53 (m, 2H, H-5 and H-8), 8.96 (s, 1H, H-2). CI MS m/z (rel. intensity) 348 (M+H$^+$, 100). Anal. Calc. for $C_{16}H_{13}NOSSe$: C 55.49, H 3.78, N 4.04. Found: C 55.31, H 3.86, N 4.08.

4.2.2.9. 3-(4-Hydroxy-2-butynylthio)-4-(propargylthio)quinoline (15). Yield 74%; mp 114–116 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.09 (t, J = 2.7 Hz, 1H, CH), 3.70 (d, J = 2.7 Hz, 2H, SCH_2), 3.87 (t, J = 2.1 Hz, 2H, SCH_2), 4.24 (t, J = 2.1 Hz, 2H, CH_2OH), 7.62–7.72 (m, 2H, H-6 and H-7), 8.05–8.58 (m, 2H, H-5 and H-8), 8.97 (s, 1H, H-2). CI MS m/z (rel. intensity) 300 (M+H$^+$, 100). Anal. Calc. for $C_{16}H_{13}NOS_2$: C 64.19, H 4.38, N 4.68. Found: C 64.32, H 4.26, N 4.76.

4.2.2.10. 4-(4-Bromo-2-butynylseleno)-3-(propargylthio)quinoline (16). Yield 53%; mp 93–94 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.28 (t, J = 2.7 Hz, 1H, CH), 3.64–3.67 (m, 4H, $SeCH_2$ and CH_2Br), 3.83 (d, J = 2.7 Hz, 2H, SCH_2), 7.61–7.74 (m, 2H, H-6 and H-7), 8.08–8.53 (m, 2H, H-5 and H-8), 8.98 (s, 1H, H-2). CI MS m/z (rel. intensity) 412 (M+2+H$^+$, 78), 410 (M+H$^+$, 100). Anal. Calc. for $C_{16}H_{12}BrNSSe$: C 46.96, H 2.96, N 3.42. Found: C 46.84, H, 2.92, N 3.51.

4.2.2.11. 4-(4-Hydroxy-2-butynylseleno)-3-(propargylthio)-quinoline (17). Yield 57%; mp 104–105 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.28 (t, J = 2.9 Hz, 1H, CH), 3.62 (t, J = 2.1 Hz, 2H, $SeCH_2$), 3.82 (d, J = 2.9 Hz, 2H, SCH_2), 4.03 (br s, 2H, CH_2OH), 7.60–7.74 (m, 2H, H-6 and H-7), 8.07–8.54 (m, 2H, H-5 and H-8), 8.98 (s, 1H, H-2). CI MS m/z (rel. intensity) 348 (M$^+$, 100). Anal. Calc. for $C_{16}H_{13}NOSSe$: C 55.49, H 3.78, N 4.04. Found: C 55.30, H 3.91, N 4.11.

4.2.2.12. 4-(4-Hydroxy-2-butynylthio)-3-(propargylthio)quinoline (18). Yield 63%; mp 108–110 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.28 (t, J = 2.6 Hz, 1H, CH), 3.73 (t, J = 2.1 Hz, 2H, SCH_2), 3.84 (d, J = 2.6 Hz, 2H, SCH_2), 4.03 (br s, 2H, CH_2OH), 7.62–7.74 (m, 2H, H-6 and H-7), 8.09–8.60 (m, 2H, H-5 and H-8), 9.01 (s, 1H, H-2). CI MS m/z (rel. intensity) 300 (M+H$^+$, 100). Anal. Calc. for $C_{16}H_{13}NOS_2$: C 64.19, H 4.38, N 4.68. Found: C 64.35, H 4.53, N 4.56.

4.2.2.13. 4-(4-Hydroxy-2-butynylseleno)-3-methylthioquinoline (19). Yield 73%; mp 115–117 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.67 (s, 3H, SCH_3), 3.61 (t, J = 2.4 Hz, 2H, $SeCH_2$), 4,01 (br s, 2H, CH_2OH), 7.61–7.67 (m, 2H, H-6 and H-7), 8.04–8.54 (m, 2H, H-5 and H-8), 8.76 (s, 1H, H-2). CI MS m/z (rel. intensity) 324 (M+H$^+$, 100). Anal. Calc. for $C_{14}H_{13}NOSSe$: C 52.18, H 4.07, N 4.35. Found: C 52.30, H 3.96, N 4.28.

4.2.2.14. 4-(4-Hydroxy-2-butynylthio)-3-methylthioquinoline (20). Yield 53%; mp 119–120 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.68 (s, 3H, SCH_3), 3.71 (t, J = 2.1 Hz, 2H, SCH_2), 4.01 (br s, 2H, CH_2OH), 7.59–7.70 (m, 2H, H-6 and H-7), 8.06–8.59 (m, 2H, H-5 and H-8), 8.80 (s, 1H, H-2). CI MS m/z (rel. intensity) 276 (M+H$^+$,100). Anal. Calc. for $C_{14}H_{13}NOS_2$: C 61.06, H 4.76, N 5.09. Found: C 61.19, H 4.64; N 5.06.

4.2.2.15. 1,4-Bis-(3-propargylthio-4-quinolinylthio)-2-butyne (21). Yield 45%; mp 145–146 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.26 (t, J = 2.4 Hz, 2H, 2× CH), 3.46 (s, 4H, 2× SCH_2), 3.79 (d, J = 2.4 Hz, 4H, 2× SCH_2), 7.55–7.72 (m, 4H, 2× H-6 and 2× H-7), 8.07–8.43 (m, 4H, 2× H-5 and 2× H-8), 8,97 (s, 2H, 2× H-2). CI MS m/z (rel. intensity) 513 (M+H$^+$, 14), 270 (19), 232 (100). Anal. Calc. for $C_{28}H_{20}N_2S_4$: C 65.59, H 3.93, N 5.46. Found: C 65.32, H 4.06, N 5.61.

4.2.2.16. 1,4-Bis-[3-(2-butynylthio)-4-quinolinylthio]-2-butyne (22). Yield 49%; mp 160–161 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 1.77 (t, J = 2.4 Hz, 6H, 2× CH_3), 3.45 (s, 4H, 2× SCH_2), 3.77 (q, J = 2.4 Hz, 4H, 2× SCH_2), 7.53–7.70 (m, 4H, 2× H-6 and 2× H-7), 8.07–8.42 (m, 4H, 2× H-5 and 2× H-8), 8.96 (s, 2H, 2× H-2). CI

MS *m*/*z* (rel. intensity) 541 ($M+H^+$, 16), 296 (12), 246 (100). Anal. Calc. for $C_{30}H_{24}N_2S_4$: C 66.63, H 4.47, N 5.18. Found: C 66.48, H 4.38, N 5.07.

4.2.2.17. 1-[3-(2-Butynylthio)-4-quinolinylseleno]-4-(3′-propargylthio-4′-quinolinylthio)-2-butyne (23). Yield 68%; mp 134–135 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.77 (t; J = 2.4 Hz, 3H, CH_3), 2.26 (t, J = 2.7 Hz, 1H, CH), 3.35 (t, J = 2.4 Hz, 2H, $SeCH_2$), 3.48 (t, J = 2.7 Hz, 2H, SCH_2), 3.76 (t, J = 2.4 Hz, 2H, SCH_2), 3.80 (q, J = 2.4 Hz, 2H, SCH_2), 7.53–7.72 (m, 4H, 2× H-6 and 2× H-7), 8.04–8.45 (m, 4H, 2× H-5 and 2× H-8), 8.94 (s, 1H, H-2), 8.98 (s, 1H, H-2). CI MS *m*/*z* (rel. intensity) 575 ($M+H^+$, 12), 294 (46), 232 (100). Anal. Calc. for $C_{29}H_{22}N_2S_3Se$: C 60.72, H 3.87, N 4.88. Found: C 60.54, H 4.01, N 4.96.

4.2.2.18. 1,4-Bis-(3-methylthio-4-quinolinylthio)-2-butyne (24). Yield 70%; mp 180–181 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.65 (s, 6H, 2× SCH_3), 3.44 (s, 4H, 2× SCH_2), 7.56–7.70 (m, 4H, 2× H-6 and 2× H-7), 8.11–8.42 (m, 4H, 2× H-5 and 2× H-8), 8.75 (s, 2H, 2× H-2). CI MS *m*/*z* (rel. intensity) 465 ($M+H^+$, 74), 258 (18), 208 (100). Anal. Calc. for $C_{24}H_{20}N_2S_4$: C 62.04, H 4.34, N 6.03. Found: C 62.20, H 4.27, N 5.94.

4.3. Antiproliferative assay in vitro

4.3.1. Cells

The following established in vitro cancer cell lines were applied: SW707 (human colorectal adenocarcinoma), CCRF/CEM (human leukemia), P388 (mouse leukemia), and B16 (mouse melanoma). All lines were obtained from the American Type Culture Collection (Rockville, Maryland, USA) and maintained at the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, USA) at a density of 10^4 cells per well in 100 μl of culture medium. The cells were cultured in the opti-MEM medium supplemented with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 μg/ml), penicillin (50 U/ml) (both antibiotics from Polfa, Tarchomin, Poland), and 5% fetal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37 °C in humid atmosphere saturated with 5% CO_2.

4.3.2. SRB assay

The details of this technique were described by Skehan.[21] The cytotoxicity assay was performed after 72-h exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich–Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (4×) with 1% acetic acid. The protein–bound dye was extracted with 10 mM unbuffered Tris base (POCh, Gliwice, Poland) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland). Each compound in given concentration was tested in triplicates in each experiment, which was repeated 3–5 times.

4.3.3. MTT assay

This technique was applied for the cytotoxicity screening against mouse leukemia cells growing in suspension culture. An assay was performed after 72-h exposure to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. For the last 3–4 h of incubation, 20 μl of MTT solution was added to each well (MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; stock solution: 5 mg/ml). The mitochondria of viable cells reduce the pale yellow MTT to a navy blue formazan: the more viable cells are present in well, the more MTT will be reduced to formazan. When incubation time was completed, 80 μl of the lysing mixture was added to each well (lysing mixture: 225 ml dimethylformamide, 67.5 g sodium dodecyl sulfate and 275 ml of distilled water). After 24 h, when formazan crystals had been dissolved, the optical densities of the samples were read on an Multiskan RC photometer at 570 nm wavelength.

Each compound in given concentration was tested in triplicates in each experiment, which was repeated 3–5 times.

The results of cytotoxic activity in vitro were expressed as an ID_{50}—the dose of compound (in μg/ml) that inhibits proliferation rate of the tumor cells by 50% as compared to the control untreated cells.

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References

[*] **Part CVII in the series of Azinyl Sulfides**

1. Nicolaou, K.; Dai, W.-M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1387.
2. Grissom, J. W.; Gunawardena, G. U.; Klingberg, D.; Huang, D. *Tetrahedron* **1996**, *52*, 6453.
3. Jones, G. B.; Fouad, F. S. *Curr. Pharm. Des.* **2002**, *8*, 2415.
4. Gredicak, M.; Jeric, I. *Acta Pharm.* **2007**, *57*, 133.
5. Davies, J.; Wang, H.; Taylor, T.; Warabi, K.; Huang, X.-H.; Andersen, R. J. *Org. Lett.* **2005**, *7*, 5233.
6. Lo, Y. H.; Lin, I. L.; Lin, C. F.; Hsu, C. C.; Yang, S. H.; Lin, S. R.; Wu, M. J. *Bioorg. Med. Chem.* **2007**, *15*, 4528.
7. Banti, D.; Groaz, E.; North, M. *Tetrahedron* **2004**, *60*, 8043.
8. Joshi, M. C.; Bisht, G. S.; Rawat, D. S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3226.
9. Tuntiwechapikul, W.; David, W. M.; Kumar, D.; Salazar, M.; Kerwin, S. M. *Biochemistry* **2002**, *41*, 5283.
10. Wu, M.-J.; Lin, C.-F.; Chen, H.-T.; Duh, T.-H.; Wang, S.-S.; Hsu, S.-C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2183.
11. Kumar, D.; David, W. M.; Kerwin, S. M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2971.
12. Rawat, D. S.; Benites, P. J.; Incarvito, C. D.; Rheingold, A. L.; Zaleski, J. M. *Inorg. Chem.* **2001**, *40*, 1846.
13. Boryczka, S.; Wietrzyk, J.; Nasulewicz, A.; Pełczyńska, M.; Opolski, A. *Pharmazie* **2002**, *57*, 733.
14. Boryczka, S.; Wietrzyk, J.; Opolski, A. *Pharmazie* **2002**, *57*, 151.
15. Mól, W.; Naczyński, A.; Boryczka, S.; Wietrzyk, J.; Opolski, A. *Pharmazie* **2006**, *61*, 742.
16. Boryczka, S.; Kulig, K.; Malawska, B. J. *Planar Chromatogr.* **2003**, *16*, 117.
17. Bajda, M.; Boryczka, S.; Wietrzyk, J.; Malawska, B. *Biomed. Chromatogr.* **2007**, *21*, 123.
18. Maślankiewicz, A.; Boryczka, S. J. *Heterocycl. Chem.* **1993**, *30*, 1623.
19. Machinek, R.; Lüttke, W. *Synthesis* **1975**, *255*.
20. Almenningen, A.; Bastiansen, O.; Tretterberg, M. *Acta Chem. Scand.* **1961**, *15*, 1557.
21. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; Mcmachon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyol, M. R. J. *Natl. Cancer. Inst.* **1990**, *82*, 1107.